

Abstracts

in diab CHOP-10KO compared to WT diab, 21 days after ischemia. This effect was associated with a reduction in the number of apoptotic cells and an increase in eNOS levels in diab CHOP-10KO compared to WT diab. We next analyzed the role of CHOP-10 in post-natal vasculogenesis. Injection of BMC isolated from diab CHOP-10KO in WT mice with hindlimb ischemia improved neovascularization by around 1.8-fold when compared to WT diab BMC ($p < 0.05$). BMC isolated from diab CHOP-10KO mice showed an upregulation of eNOS protein levels and an increase in their ability to differentiate into cells with endothelial phenotype in vitro differentiation assay. Finally, treatment of cultured HUVEC with homocysteine increased CHOP-10 mRNA levels and repressed eNOS gene expression. Consistent with these results, eNOS protein expression was significantly upregulated in the CHOP-10 siRNA-transfected endothelial cells. Additionally, overexpression of CHOP-10 inhibited the basal transcriptional activation of the eNOS promoter as assessed by a reporter gene assay using a 3,500-bp fragment of the human eNOS gene.

Our study unravels an important inhibitory role of CHOP-10 in the regulation of vessel formation in the setting of diabetes.

D024

LYSYL OXIDASE LIKE 2 REGULATES VASCULAR CELLS MIGRATION AND BASAL LAMINA ORGANISATION

M. BIGNON¹, J. HARDOUIN², N. BRECHOT¹, L. NASCIUTTI³, R. JOUBERT-CARON², M. CARON², S. GERMAIN^{1,4}, C. MONNOT¹, L. MULLER¹

¹ Inserm U833 – Collège de France, PARIS, FRANCE, ² LBPP – UMR CNRS 7033 Université Paris XIII, Bobigny, France

³ Universidade Federal do Rio de Janeiro, Rio De Janeiro, Brazil

⁴ Service d'Hématologie Biologique A – Hôpital Européen Georges Pompidou – AP-HP, Paris, France

Hypoxia stimulates angiogenesis during development, as well as in the course of ischemic cardiovascular diseases and tumor growth. In a hypoxic environment, endothelial cells (EC) are key players of the angiogenic response. Their activation leads to remodeling of the extracellular matrix (ECM): a degradation step generates a provisional ECM supporting EC proliferation and migration; assembly of a new basal lamina leads to pericyte recruitment and neovessel maturation.

In order to identify endothelial ECM proteins regulated by hypoxia, 2D gel electrophoresis was performed on ECM samples prepared from cultured EC of micro or large vessels (HDMEC or HUVEC respectively). Mass spectrometry identified lysyl oxidase-like protein 2 (LOXL2) as a highly hypoxia-induced protein. Lysyl oxidases are secreted enzymes involved in ECM maturation through covalent cross-linking of its major components, collagens and elastin. The induction of LOXL2 expression was detected both at the mRNA and protein levels. Using siRNA, we demonstrated that LOXL2 is responsible for 65% of lysyl oxidase total activity in hypoxic EC. In addition, LOXL2 protein was colocalised with type IV collagen in endothelial ECM.

We further investigated the expression of LOXL2 in vivo. The enzyme was expressed in rat EC from retina during postnatal vascular development, and from adult skeletal muscle. In a murine model of hindlimb ischemia, LOXL2 was upregulated at the protein and mRNA levels. In situ hybridization revealed its expression in both EC and macrophages.

Involvement of LOXL2 at different steps of the angiogenic process was further studied in vitro. We demonstrated that LOXL2 increases EC migration on fibronectin, as well as migration and tube formation in fibrin 3D gels. In addition, LOXL2 knock-down inhibited type IV collagen deposition in the ECM, suggesting a major role for LOXL2 in the organisation of endothelial basal lamina. Finally, whereas the efficiency of endothelial ECM for recruiting VSMC was increased by hypoxia, this effect was reduced upon knocking down endothelial LOXL2 expression. Altogether, these data suggest that LOXL2 plays a major role in EC migration, vascular basal lamina deposition and neovessel stabilisation during hypoxia-induced angiogenesis.

D025

PHAGOCYTOSIS IS PIVOTAL IN THE BENEFICIAL EFFECT OF BONE MARROW MONONUCLEAR CELLS-BASED THERAPY FOR MYOCARDIAL INFARCTION

C. COCHAIN¹, P. BONIN¹, J. VILAR¹, A. RÉCALDE¹, Y. ZOUGGARI¹, C. LOINARD¹, A. RICHART¹, V. POURSMAIL¹, M. DURIEZ¹, B. LÉVY¹, Z. MALLAT¹, J.-S. SILVESTRE¹

¹ Inserm U689, Paris, France

Cell-based therapy is a promising option for treatment of cardiovascular diseases. Based on experimental studies demonstrating that bone marrow-derived mononuclear cells (BMMNCs) improve the functional recovery after ischemia, clinical trials were initiated to address this new therapeutic concept. BMMNCs improve neovascularization of ischemic tissue by a broad repertoire of potential therapeutic actions. Whereas initial studies documented that the cells incorporate and differentiate to cardiovascular cells, other studies suggested that short-time paracrine mechanisms mediate the beneficial effects. Here, we hypothesized that BMMNCs have a phagocytic ability, and switch to a proangiogenic phenotype after engulfment of apoptotic cells. Activation of such angiogenic program may be pivotal in the beneficial effect of BMMNCs-based therapy. In vitro, wild-type (WT) BMMNCs ingestion of apoptotic cells upregulated the release of proangiogenic factors VEGF and HGF by 15- and 5-fold, respectively. In contrast, BMMNCs collected from mice deficient in MFG-E8, a protein that is required for attachment and engulfment of apoptotic cells by phagocytes, displayed lower phagocytic ability, leading to decrease in VEGF and HGF release. The capacity of BMMNCs to differentiate into cells with endothelial phenotype was similar in control and MFG-E8-deficient cells. In an in vivo model of mice myocardial infarction (MI), transplantation of WT BMMNCs increased fractional shortening (120% of untreated control, $p < 0.05$), 14 days after MI. Size of the infarct scar was reduced by 30% ($p < 0.05$ vs untreated control), and capillary density in the infarct border zone was raised by 10% ($p < 0.05$ vs untreated control) in the WT BMMNCs group. In contrast, transplantation of MFG-E8 deficient BMMNCs displayed no significant effect on cardiac function, infarct size or angiogenesis in the ischemic myocardium. Four days after MI, VEGF protein levels were raised by 1.4 fold in the myocardium of WT BMMNCs treated animals compared to untreated controls ($p < 0.05$), while treatment with MFG-E8 deficient BMMNCs failed to raise VEGF levels. Taken together, these results suggest that phagocytosis of apoptotic cells reprograms BMMNCs into a proangiogenic phenotype. Hence, the therapeutic effect of transplanted BMMNCs depends, at least in part, on their phagocytic ability.